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Rothamsted Experimental Station, Harpenden, Herdfordshire, England

Studies in leaf litter breakdown
III. The influence of leaf age

C. A. Edwards and G. W. Heath

With 5 figures

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1. Introduction

The importance of soil invertebrates in the breakdown of leaf litter has been demonstrated (Edwards and Heath 1963; Witkamp and Crossley 1966) by using techniques of confining batches of 2.5 cm diam leaf-disks in small nylon-mesh bags and burying them in soil or litter. The size of the mesh from which the bag was made determined the size of animal that could feed on the litter and showed that species with tougher leaves did not break down unless they were first disintegrated by invertebrate feeding. The loss of tissue due to the feeding of invertebrates could be assessed by measuring changes in surface area by a photometric technique (Heath et al. 1964), and the loss due to both invertebrates and microorganisms could be measured from the loss in weight of the same leaf disks.

Early in the investigations, it became obvious that some beech and oak leaf disks broke down much more readily than others, and that this was correlated with the original position of the leaves on the tree, so that they could be categorized as either "sun" or "shade" leaves (Heath and Arnold 1966). It was later shown that the more rapid degradation of "shade" leaves could be inversely correlated with the amount of watersoluble polyphenolic compounds they contained (King and Heath 1967); for instance, Satchell and Lowe (1967) showed that water-soluble polyphenols could influence the palatability of leaf tissues to earthworms. Feeny and Bostock (1968) demonstrated that there were gradual increases in the phenolic content of oak (*Quercus robur* L.) leaves on the tree throughout the growing season, and they suggested that this might be why insects attack mature tree leaves much less than young ones. In this paper, the possibility that the gradual increase in polyphenols in leaves determines their palatability to soil and litter invertebrates is assessed.

2. Materials and Methods

More than 250 oak leaves and 500 beech leaves were collected from medium-sized trees approximately every two weeks from 17 June to 21 October 1964, and from the leaf litter in November and December 1964 (13 samples in all). Leaf disks 1″ (2.5 cm) in diameter were cut from the leaves using a special device (Heath et al. 1964). They were surface-sterilized by dipping in a 1:1500 solution of "Nipagin" (methyl parahydroxybenzoate) and exposing them under a germicidal ultraviolet lamp, and then packed tightly, in heat-sealed polythene bags with no enclosed air, and stored at 2—3 °C until required.

One hundred and four nylon bags (0.7 cm aperture mesh, "large") and 52 (0.15 cm aperture mesh, "small") each measuring 10×10 cm were made by machine sewing round the edges with nylon thread.

On 17 December 1964, 400 beech and 200 oak disks cut from leaves collected on each of the thirteen sampling dates were selected at random from those in store. Fifty beech disks were placed in each of four large-mesh bags, 50 beech in each of four small-mesh bags and 50 oak disks in each of four large-mesh bags, giving a total of 52 large-mesh bags containing oak disks, 52 containing beech and 52 small-mesh bags containing beech. The bags were labelled with plastic tabs and numbered according to the code numbers in Table 1. All bags were buried in a random pattern at a depth of 2.5 cm below the liter layer in an oak-dominant mixed woodland (EDWARDS and HEATH 1963).

Table 1. Dates leaves were collected

Code	Date of collection (1964)	Source of leaves	Condition of leaves when buried	
			Oak	Beech
A	17 June	tree	Brownish/green	dark green
B	30 June	tree	Brownish/green	dark green
C	15 July	tree	Brownish/green	dark green
D	29 July	tree	Brownish/green	dark green
E	12 August	tree	Brownish/green	dark green
F	26 August	tree	Brownish/green	dark green
G	9 September	tree	Brownish/green	dark green
H	24 September	tree	Brownish/green	dark green
I	7 October	tree	Brownish/green	dark green
J	21 October	tree	Yellowish/brown	brown
K	5 November	litter	Brown	brown
L	20 November	litter	Brown	brown
M	3 December	litter	Brown	brown

At intervals ranging from 2 to 3 months (see Tables 2, 3 and 4) the overlying leaf litter was cleared and the bags were dug up, brought to the laboratory and kept at 2—3 °C whilst assessments were being made. The assessments of the amounts of litter consumed were made by opening each mesh bag in turn laying the 50 disks out on a glass plate in a 20 cm square covering this with another glass plate and measuring the amount of leaf tissue that had disappeared using a specially constructed photometer (HEATH et al. 1964). Once an assessment had been made the disks were returned to the bags, which were closed with copper staples, and kept at 2—3 °C until they could be reburied.

3. Results and Discussion

The results are summarized in tables 2—4 and figs. 1—4. The Tables give actual data for sampling dates, with statistical analysis. Data from pairs of sampling dates have been bulked for the breakdown curves (figs. 1, 2 and 3) to avoid confusion and clarify the picture. Statistically the amounts of tissue that had disappeared differed significantly between leaf disks of different ages for all assessment dates except the first, and, even on this assessment, the amounts that had disappeared from the beech disks in small-mesh bags differed significantly between disks of different ages. Throughout the experiment the variability in estimates from the small-mesh beech bags for any single date both within bags and between replicates was much less than from either oak or beech in large-mesh bags. This is probably because a large number of small animals were feeding on the disks in small-mesh bags, whereas fewer large animals, one of which could consume a relatively large proportion of the disks in any one bag, fed on the disks in the large-mesh bags.

The data (figs. 1, 2 and 3 and tables 2 and 3) confirmed the conclusions of EDWARDS and HEATH (1963) and EDWARDS et al. (1970) that the larger animals in the litter could consume several times as much oak and beech leaf tissue (irrespective of age) as could the micro- and meso-fauna. It has been reported that earthworms consume two thirds of the litter fall in temperate zones, macroarthropods accounting for most of the rest, with mites and springtails disintegrating only a few per cent of the total (EDWARDS 1974).

There may be a bias in our data to overestimating the amount of litter consumed by the larger invertebrates in the large-mesh bags because, occasionally, an earthworm or larger arthropod became trapped within one of these bags and consumed large amounts. Moreover, earthworms sometimes pulled whole disks from a large-mesh bag and took them down their burrows; such disks would be assessed as 100% loss in weight or surface area but in fact would not be completely broken down.

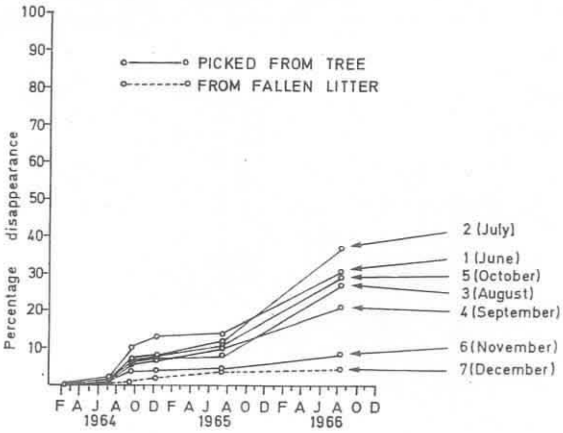

Fig. 1. Breakdown of beech leaf disks in 0.1 mm mesh bags.

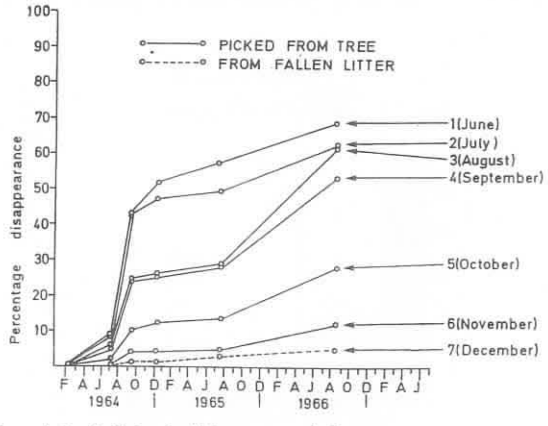

Fig. 2. Breakdown of beech leaf disks in 0.7 mm mesh bags.

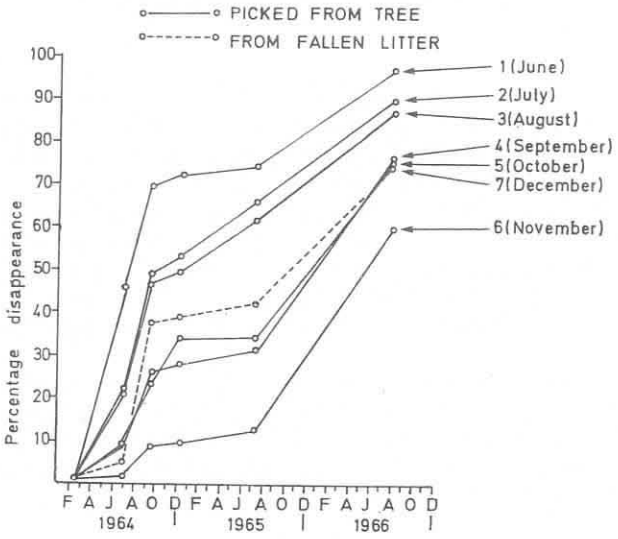

Fig. 3. Breakdown of oak leaf disks in 0.7 mm mesh bags.

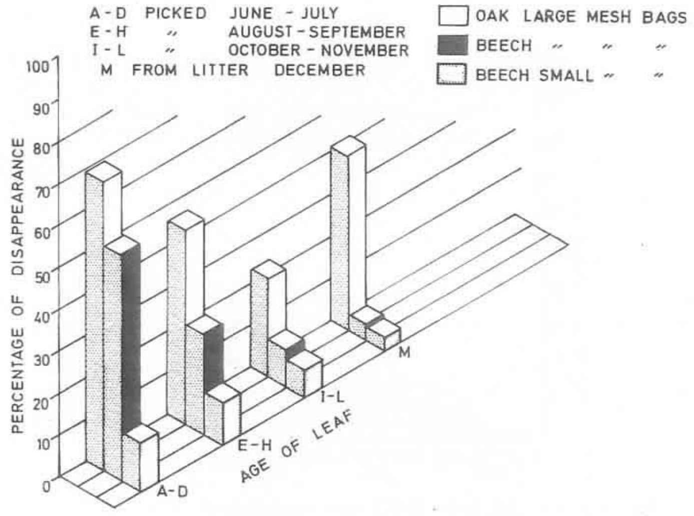

Fig. 4. Amounts of leaf tissue disappearing after 18 month exposure to soil animals.

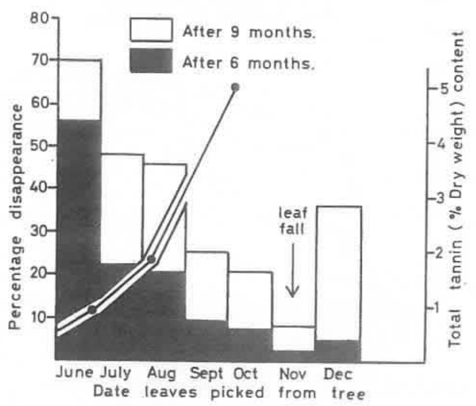

Fig. 5. Relationship between age of leaf, percentage disappearance and tannin content (in small mesh bags under litter).

It was not possible to assess completely which animals were consuming the litter disks in the two kinds of mesh bags, but the animals most commonly found in the small-mesh bags were springtails, especially onychiurids, oribatid mites and enchytraeid worms. In the large-mesh bags these animals were also found, together with millipedes, dipterous larvae and earthworms; of these groups earthworms seemed to be consuming by far the largest amounts.

The most interesting feature of the data is that they demonstrate clearly that as the leaves on a tree become older they gradually become less palatable to soil animals just as they do to those arthropods that feed on them whilst they are still on the tree; moreover, whatever it is that makes the older leaves less palatable persists even after the leaves have fallen from the tree.

As reported previously (Edwards and Heath 1963; Heath and Arnold 1966; Heath et al. 1966), oak leaves broke down much more rapidly than beech leaves. There seemed to be one basic difference between the ageing of oak and beech leaves; whereas beech became progresively less palatable throughout the growing season and continued to be so even after several weeks in the leaf litter (figs. 2, 3 and 4, tables 2 and 3), oak, although it became

Table 2. Percentages of leaf tissue lost with time — analysis of variance beech — fine mesh bags

Age of leaf date	June		July		August		September	
	A	B	C	D	E	F	G	H
3. 6. 1964	2.38	1.5	1.0	0.73	0.23	0.33	0.55	0.13
16. 9. 1964	8.0	12.0	6.0	5.67	5.25	6.0	7.0	4.5
18. 12. 1964	10.0	16.17	7.67	6.33	6.13	7.33	8.13	5.0
19. 7. 1965	10.13	17.5	10.0	7.67	6.0	7.83	8.0	16.38
5. 8. 1966	20.75	35.67	40.67	51.0	31.0	23.0	20.25	22.0

* = Unequal replication — modified Tukey's Test used.

Table 3. Percentages of leaf tissue lost with time — analysis of variance

Age of leaf date	June		July		August		September	
	A	B	C	D	E	F	G	H
18. 2. 1964	0.0	0.25	0.13	0.0	0.13	0.03	0.05	0.0
3. 6. 1964	7.63	4.33	11.33	5.67	9.0	3.33	4.88	4.28
16. 9. 1964	42.75	41.33	42.0	43.0	41.0	8.33	21.0	27.25
18. 12. 1964	47.25	56.0	48.33	45.33	40.25	8.67	21.5	30.5
19. 7. 1965	46.75	60.67	48.0	46.0	41.25	11.33	23.38	32.5
5. 8. 1966	67.75	71.67	61.67	60.67	71.75	53.67	49.5	57.25

* = Unequal replication — modified Tukey's Test used.

Table 4. Percentages of leaf tissue lost with time — analysis of variance oak — large mesh bags

Age of leaf date	June		July		August		September	
	A	B	C	D	E	F	G	H
18. 2. 1964	0.93	1.33	0.23	0.38	0.55	0.03	0.30	0.08
3. 6. 1964	77.25	21.75	31.13	12.0	36.0	4.5	9.75	6.0
16. 9. 1964	86.75	51.0	74.25	22.75	58.75	33.5	29.75	21.75
18. 12. 1964	92.00	52.13	80.25	25.75	65.5	32.75	31.25	22.0
19. 7. 1965	90.25	51.75	80.25	26.75	65.0	38.0	34.0	27.25
5. 8. 1966	99.0	93.25	95.0	84.25	85.25	88.0	76.5	73.5

gradually less palatable whilst on the tree, it was much more palatable to the soil animals once it was in the litter and became weathered (fig. 1). This was confirmed in laboratory experiments, in which newly-fallen oak and beech leaves were artificially weathered by soaking them in slowly running water for a week and then offering them to soil animals as food. Earthworms and arthropods consumed such artificially weathered oak leaves very much faster than unleached ones, but leached beech leaves were eaten only slightly more quickly. However, it seems likely from later, unpublished work, that continued weathering for several months gradually makes beech leaves more palatable, probably by a combination of softening the tissues and leaching of some polyphenols.

It has been shown clearly (Handley 1961; Feeny 1968) that there is a considerable increase in polyphenolic materials in leaves between June and September; these precipitate protein complexes and would make them less digestible. Such changes would fit well with the results on changed palatability reported here. Fig. 5 shows the relationship between the age of leaves and their percentage disappearance and on the same graph Feeny's data for the seasonal increase in total tannins (polyphenols) in oak leaves have been plotted. The inverse correlation taken in conjunction with the work of King and Heath (1967) seems

October		November		Dec.	Standard error	Least significant difference	Tukey's D 0.05	F
I	J	K	L	M				
0.0	0.5	0.0	0.0	0.0	0.54	1.10	+	3.43**
5.25	5.75	3.0	4.0	0.5	2.17	4.43	+	4.03**
7.25	8.75	3.5	4.53	1.63	2.91	5.94	+	3.91**
9.0	10.88	4.0	5.17	2.6	3.66	7.47	+	4.0**
28.0	30.25	11.5	6.0	4.67	6.80	13.89	+	9.18**

October		November		Dec.	Standard error	Least significant difference	Tukey's D 0.05	F
I	J	K	L	M				
0.05	0.0	0.0	0.0	0.03	0.12	0.24	0.41	0.81
3.13	1.13	0.0	0.17	0.13	3.23	6.60	+	2.44**
16.25	4.25	5.67	3.33	1.5	11.14	22.75	+	5.08**
18.0	6.5	5.33	3.33	1.38	10.04	20.50	+	8.01**
19.0	8.0	6.0	3.0	2.25	10.41	21.26	+	7.68**
38.0	18.0	18.0	6.67	5.0	12.22	24.95	+	10.08**

October		November		Dec.	Standard error	Least significant difference	Tukey's D 0.05	F
I	J	K	L	M				
1.13	0.13	0.03	0.03	0.0	0.48	0.97	1.69	1.77
11.75	7.75	1.5	0.85	4.75	7.30	14 75	25.71	16.66**
40.25	24.5	10.5	6.75	37.5	10.36	20.94	36.48	10.55**
42.75	27.0	11.0	7.25	38.0	10.13	20.47	35.66	12.79**
47.48	29.25	12.5	12.38	41.75	10.02	20.25	35.27	11.42**
82.75	74.5	56.5	62.5	73.0	10.00	20.22	35.23	3.09**

to be good enough evidence to indicate that polyphenols are a major factor influencing palatability of leaf tissues to soil animals. Other experiments not reported here have shown that addition of polyphenolic materials to otherwise palatable leaves makes them unpalatable.

4. Zusammenfassung

Untersuchungen des Streuabbaues. I. Einfluß des Blattalters

Der statistisch nachweisbare Rotteschwund von Blättern verschiedenen Alters ist signifikant verschieden. Das gilt für alle Schätzwerte, mit Ausnahme der ersten, und gerade die Schätzwerte für die Schwundraten von Buchenblättern in engmaschigen Gazebeuteln sind in Abhängigkeit vom Alter der Blätter signifikant verschieden. Insgesamt war die Variabilität der Schätzungen innerhalb des Versuches mit Buchenblättern in engmaschigen Gazebeuteln für alle Einzelwerte sowohl innerhalb der Parallelreihen (engmaschige bzw. weitmaschige Gazebeutel) als auch zwischen den Wiederholungen bei Eichen- und Buchenblättern in den weitmaschigen Gazebeuteln weit geringer. Dies liegt wahrscheinlich daran, daß in den engmaschigen Gazebeuteln eine große Zahl von kleinen Tieren (mit geringer Fraßleistung) frißt, während eine relativ geringe Anzahl großer Tiere, mit einer relativ hohen Fraßleistung, am Rotteschwund in den weitmaschigen Gazebeuteln beteiligt ist.

Die gewonnenen Daten bestätigen (s. Abb. 1, 2 und 3 sowie Tab. 2 und 3) die Schlußfolgerungen von Edwards und Heath (1963) und Edwards et al. (1970), nach denen die größeren Tiere im Vergleich zur Mikro- und Mesofauna das Mehrfache an Eichen- und Buchenblättern (jeglichen Alters) konsumieren können. Das Interessanteste an den vorliegenden Daten ist der klare Beweis, daß das Blattalter die Genießbarkeit der Blätter für die Bodentiere in gleicher Weise beeinflußt wie für andere blattfressende Arthropoden. Das, was die älteren Blätter weniger genießbar macht, überdauert auch den Laubfall.

Von anderen Autoren (Handley 1961, Feeny 1968) wurde klar gezeigt, daß zwischen Juni und September der Polyphenolgehalt in den Blättern erheblich ansteigt; dadurch wird der Proteinkomplex präzipitiert und die Blätter werden weniger verdaulich (s. dazu Abb. 5). Andere, hier nicht erwähnte Experimente zeigten, daß Zusätze von polyphenolischen Materialien sonst genießbare Blätter (für Bodentiere) ungenießbar machen.

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Address of the authors: Dr. C. A. Edwards, Entomology Department, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ and Dr. G. W. Heath, Institute for Marine Environmental Research, 67/69 Citadel Road, Plymouth, Devon, U.K.